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REVIEW ARTICLE.....

Cytogenetical techniques for improvement of cattle

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INTRODUCTION.....

The animal genetic resources of India are represented by a broad spectrum of livestock. The genetic characterization of synthetic crossbred cattle is a major prerequisite in its conservation as well as improvement programmes. It helps in confirmation of the species of origin.

Cytogenetics is the correlated study of genetics and cytology. Relationship of specific genes with specific chromosomes is actually science of cytogenetics. It basically involves study of chromosome structure and behaviour. Animal cytogenetics is useful for enhancing reproductive performance, genetic capability, management and care in health, diseases of all classes of domestic animals.

The karyotype of a population of cells or an organism is the catalogue of the chromosomes of a typical or an average cell. Karyotyping is a useful technique in experimental biology. It can help in screening the breeding bulls for existence of hereditary diseases caused by detectable chromosome effects, significantly when such bulls are used in the artificial insemination programmes.

Chromosome identification has traditionally been dependent on their morphological characteristics such as relative lengths, arm ratios and presence or absence of secondary constrictions. However, the development of chromosome banding techniques during the last more than two decades provided a very useful additional tool for identification of individual chromosomes within the complement. The stains instead of uniformly dying the entire chromosome display characteristic bands. Individual chromosome can be observed for detection of abnormalities if any. Banding patterns also permit to establish a correlation between linkage maps and cytological maps. Modern cytogenetic studies find application in understanding heredity mechanisms, transmission of genetic diseases, cytotaxanomy and characterization of genetic resources.

Theory of chromosome :

Chromosome:

Darkly stained bodies of nucleus, capable of self reproduction and play vital role in heredity, mutation, variation and evolutionary development of the species.

Chromosome number:

Remains constant for particular species. E.g. cattle (2n=60).

Morphology :

Size : The size of chromosome is normally measured at mitotic metaphase.

Shape : The shape of the chromosome is changeable from phase to phase in the continuous process of cell division. At rest phase or inerphase stage of cell division they occur in the form of thin, coiled, elastic and thread like stainable structure. At metaphase they become thick and filamentous. Each chromosome contains a clear zone called as centromere along their length which divides the chromosome into two parts each part is called as chromosome arm.

Position of centromere varies from chromosome to chromosome and provides different shapes as:

Telocentric :

The rod like chromosomes have centromere on the proximal end.

Acrocentric:

The acrocentric chromosomes have the centromere at one end.

Submetacentric:

They are J or L shaped. Centromere occurs near the centre or at medium portion of the chromosome and thus forms unequal arms.

Metacentric:

The metacentric chromosomes are 'v' shaped in which centromere occurs near the centre and forms two equal arms.

Chromosome preparations are made by using short term peripheral blood lymphocyte culture technique.

Cytogenetic analysis :

Karyotype :

A karyotype represents an arrangement of chromosomes on the basis of their morphology and other characteristics. The homologous chromosomes are paired and arranged in descending order of their length from left to the right. They are rearranged with regard to their length and centromeric position. The chromosomes are finally pasted with short arm (P) on upper side with their centromere lined up along each row. The sex chromosomes are pasted separately after arranging autosomes.

A karyotype showing large differences between the smallest and the largest chromosomes of the set and having fewer metacentric chromosomes is called an asymmetric karyotype, which is considered to be a relatively advanced feature compared with symmetric karyotypes.

Idiogram :

An idiogram is essentially a diagrammatic representation of the chromosomes in which each chromosome is shown as a thick vertical bar. The chromosomes are represented on the basis of their relative length and position of centromeres. Usually only one chromosome of each homologous pair is represented in an idiogram. The bars are arranged in descending order of the relative length, the short arm being upper most.

Recording of qualitative and quantitative atributes of chromosomes :

Morphology of chromosomes :

Depends on its total length and position of centromere.

$$\text{Centromere index} = \frac{\text{Length of short arm}}{\text{Chromosome length}} \times 100$$

Table of centromeric range and chromosomal morphology (Dark)

Centromere Index range	Morphology of chromosome
46-49	Metacentric
31-45	Sub-metacentric
15-30	Acrocentric

Measurement of chromosomes :

Metaphase plates having sharp contrast and clear outlines of the chromosomes should be chosen for measurements. The measurement is recorded with the help of vernier caliper.

Diploid count :

The total numbers of chromosomes are recorded from a good somatic metaphase cell. The chromosome number with highest frequency is deemed to represent the diploid count.

Relative length of chromosomes :

The relative length indicates the relative size of the chromosome in relation to other chromosome in set.

$$\text{Relative length} = \frac{\text{The length of individual chromosome}}{\text{Total length of all the chromosomes in the haploid set including x Chromosome}} \times 100$$

Cytogenetic characterization carried in cattle breeds :

Desai *et al.* (1984) reported the average relative length of X-chromosomes in HF, RD and Jersey as 5.197±0.098, 5.53±0.112 and 4.877±0.171 per cent, respectively. In present study, no significant difference was observed between long X- chromosome of cows and X- chromosome of exotic bulls.

Pushpendra Kumar *et al.* (1995) studied karyotyping of Hariana cattle. They explained that the contribution of first pair of autosome as 5.48 per cent and last pair as 1.76 per cent to the total complement length. The X- chromosome contributed 5.30 per cent and Y- chromosome 2.04 per cent to the total haploid genome.

Choudhary *et al.* (1997) observed submetacentric X- chromosome in both Assam local male and Jersey bull. They reported the significant difference in the relative length of autosomes in Assam local male and Jersey bull.

Prakash (2003) explained that the two breeds of zebu cattle *i.e.* Dangi and Kankrej possessed a similar karyotype, having a diploid count of 60 consisting of 29 pairs of autosomes and a pair of sex chromosomes.

Ravi Kumar *et al.* (2003) analysed chromosome architecture of Punganur breed of cattle. The diploid chromosome number for the breed is 60. All the autosomes and the Y- chromosome are acrocentric, while the X- chromosome is sub-metacentric. The mean arm ratio, centromeric index and morphological index of X-chromosme are 0.56, 0.36 and 4.40, respectively.

Balaji *et al.* (2006) conducted Cytogenetic characterization of Deoni cattle. The study revealed the diploid chromosome number to be 60, comprising 29 pairs of autosomes having Y-chromosome acrocentric while X-chromosome was submetacentric. They observed the chromosome architecture of Deoni cattle similar to that of the other breeds of zebu cattle.

Suresh *et al.* (2006) compared the karyotype of Malnad Gidda cattle (*B.indicus*) with Jersey (*B.taurus*). They observed X-chromosome of both breeds was submetacentric and Y- chromosome was acrocentric in Malnad Gidda and metacentric in Jersey cattle. No significant difference was observed between Malnad Gidda and Jersey cattle in the mean relative length of chromosomes.

Chromosomal aberrations or abnormalities :

Changes in the genome involving chromosome parts, whole chromosomes or whole chromosomes sets are called as chromosome aberrations.

Structural changes in chromosomes :

Changes in chromosome number :

Loss/Deletion:

The simplest result of breakage is the loss of a part of chromosome.

Addition /Duplication:

The presence of a part of a chromosome in excess of the normal complement is known as duplication.

Genetic effect of duplication :

Due to duplication, there occur unequal crossing over which results in deletion and reduplication which produces distinct phenotypes.

Changes in gene arrangement :

Rotation of a group of genes 180^0 within one :

Chromosome/ Inversion :

It involves a rotation of a part of a chromosome or a set of genes by 180^0 on its own axis. The inversion requires two breaks along the length of chromosome prior to the reinsertion of the inverted segment. If the centromere is not a part of the rearranged chromosme segment, the inversion is called paracentric and if the centromere is a part of the inverted segment, the inversion is called pericentric.

Exchange of parts between chromosomes of different parts/ translocations :

In this category of aberration a segment of the chromosome shifts to a new place and integrates in the genome. Translocation may occur within a single chromosome or between the non-homologous chromosomes. The exchange of the segments between two non-homologous chromosomes is a type of structural

variation called as a reciprocal translocation.

Robertsonian translocation :

Sometimes whole arm fusions occur in the non-homologous chromosomes called as Robertsonian translocation. It results in a reduction of the chromosome number.

These structural rearrangements of chromosomes may lead to reproductive isolation and the formation of new species. Patel (1999) observed the young HF crossbred bull to be carrier of Robertsonian translocation, rob (7, 16) *i.e.* chromosome 7 and 16 were involved in the translocation. The bull carrying the new translocation, rob (7, 16) was phenotypically normal.

Chromatd type :

GAP (9): This type of aberration involves only one chromatid of each chromosome except for isochromatid breaks.

The cytogenetic screening of bulls carried at All India Network Project on Animal Genetic Resources. They recorded two bulls were identified as afflicted with chromosomal defects. One was sex chromosome chimeric (50, XY / 50, XX) and the other carried chromatid gaps and breaks in almost 40 per cent of its metaphase spread analysed.

Changes in number of chromosomes :

Aneuploidy:

Loss or gain of a part of the chromosome set

Euploidy:

Loss or gain of whole chromosome set

Haploidy:

Loss of an entire set of the chromosomes

Polyploidy:

Addition of one or more sets of the chromosomes.

Introduction of banding patterns :

Principle of banding :

The banding techniques are based on the identification of chromosome segments that predominantly consist of either GC or AT rich regions or the constitutive heterochromatin. The chromosomes after staining when observed under ultraviolet light them fluorescence and show the characteristic bands, these bands are distinct for each chromosome which helps in study.

It involves denatueration of DNA, followed by slow renaturation, permits identification of constitutive hererochomatin because it mainly consists of repeatative DNA. A variety of different kinds of bands (e.g. Q, C, G, R etc.) have been studied.

Q bands (Quinacrin) :

Caspersson and his coworkers demonstrated that quinacrin mustard (which is a fluorochrome and has affinity with specific DNA) staining and fluorescence in UV light, produces characteristic bright and dark bands on chromosomes. In their width, brightness and position these Q bands are so unique that individual chromosomes can be identified.

C bands (Centromeric) :

It is a general technique for staining constitutive heterochromatin demonstrated by the denaturation – reassociation techniques.

N bands :

N banding technique which was originally developed for location of nucleolar organizers (NORs) in animal chromosome.

Shashikanth *et al.* (1991) studied the nucleolar organizing regions of Amritmahal, Hallikar, Holstein Friesian and Jersey cattle by Ag-NORs staining technique. The NORs were observed on telomeric regions of 5 pairs of chromosomes. The mean number of Ag-NORs per NOR- positive cells was 5.8, 5.2, 6.1 and 5.3 in above cattle, respectively.

G bands (Giemsa) :

Instead of squash preparations air dried or flame dried preparations of lymphocyte cultures are used for G bands.

Nagpure *et al.* (2006) carried comparative C and G banding studies in Hariana, Holstein Friesian and their crossbreds. The sex chromosomes in all genetic groups were found to be C band negative, while prominent C-bands observed on all automomes. No heteromorphism was noticed for G-banding pattern among the genetic groups.

R bands :

R bands show a pattern that is reverse of G- bands *i.e*. light banded regions of G banded chromosome become darkly stained and *vice versa*. In recent years bands stained have also been detected after treatment of chromosomes with restriction enzymes (RE). These bands have been described as RE bands.

Tomar and Goswami (1999) observed the banding pattern produced by restriction endonuclease HINF I in chromosomes of Sahiwal, Tharparkar, Jersey and Holstein Friesian cattle and Murrah and Nili-Ravi buffaloes. In cattle the chromosomes after treatment with HINF I produced C-like banding pattern simultaneously with intercalary bands on chromosomal arm which seemed to resemble R-band pattern rather than G-band pattern. The chromosome was found to be C-band negative. The band pattern was similar in all the four breeds.

Advancements in chromosome analysis

In situ hybridization with DNA probes(ISH) :

In this technique we like to locate the physical position of a known DNA sequence on a chromosome. Thus the technique helps in physical mapping of genes or repeated DNA sequence. In this technique DNA within the cell is denatured by treating the cells that have been squashed on a cover slip. The squashed cells can then be incubated in a solution of labeled DNA, whose position on a chromosome, we are interested in knowing.

Fluorescence in situ hybridization (FISH) :

In the above technique of *in situ* hybridization, specific DNA sequences are located on chromosomes through the use of radioactive isotopes or through the use of non-radioactive labeling followed by staining reaction.

Computer assisted chromosome analysis (CACA):

While preparing karyotypes or ideograms', the chromosomes are not lying straight, but are curved at metaphase plate or overlapping error occurs during measurements. To overcome these difficulties, interactive computer assisted image processing systems are available with appropriate software for the purpose. By this not only the length of chromosomes and position of primary and secondary constrictions are correctly located, but even the intensity and width of different C bands are correctly measured.

Further scope of cytogenetic work :

Modern cytogenetic innovations have found numerous practical applications and can be used in future for

– Assessment of the chromosome abnormalities and their impact on the phenotype, fertility and sterility.

– In assaying the genetic damage and toxicological effects, evaluation of Cytogenetic profiles provides a reliable parameter. The sister chromatid exchanges are now considered a more sensitive parameter in the evaluation of potent genotypic, genotoxic, caroinogenic and mutagenic agents.

– Prenatal sexing of embryos prior to transfer in the embryo transfer technology technique.

– Understanding the genetic homology and diversity in the allied and related animal breeds.

– Understanding the possible mode or mechanism of an evolution and speciation.

Conclusion :

– As the chromosomes are the carriers of all hereditary or genetic information so cytogentic characterization must carried to detect chromosome abnormalities.

– These abnormalities can be eliminated by studying the individual chromosome by banding techniques which maintain the breed cytogenetically clean.

– Screening of breeding males is necessary during artificial insemination to avoid spread of diseases through chromosomes.

– It is now realized that in depth and comprehensive information on chromosomes is an essential pre requisite for all programmes in genetic biotechnology, gene mapping, gene transfer and animal breeding.

– Several significant innovations are made in protocols coupled with use of sophisticated tools and high level of automation which provides novel method for analyzing in depth with greater precision- the architecture of chromosom

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